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SOMATOTOPY IN THE REPRESENTATION OF THE PECTORAL FIN AND FREE FIN RAYS IN THE SPINAL CORD OF THE SEA ROBIN, *PRIONOTUS CAROLINUS*

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Abstract

Sea robins possess modified pectoral fin rays which are chemosensory although lacking taste buds or olfactory receptors. These fin rays are innervated only by spinal nerves which terminate in accessory spinal lobes, enlargements of the dorsal horn in the rostral spinal cord.

Horseradish peroxidase was used as a neuronal tracer to determine the representation of each fin ray nerve in the spinal cord. The nerve innervating each fin ray terminates in a single accessory lobe with the ventral fin ray terminating in the caudal accessory lobe and the dorsal fin ray in the rostral major accessory lobe. The pectoral fin itself is represented in a minor spinal enlargement lying rostral of the major accessory lobes.

Introduction

Sea robins (*Prionotus*) and the related European gurnards (*Triglida*) possess modified pectoral fins which are capable of detecting low levels of certain chemicals despite the absence of taste buds or other specialized chemosensory end organs (Whitear, 1971). In these genera, the three most ventral fin rays are free from the rest of the pectoral fin (Fig. 1). The free fin rays are moved independently of the fin and are used actively to explore the substrate (Morrill, 1895; personal observation). The fish will respond positively when the free fin rays contact food or food extracts (Bardach and Case, 1965).

Despite the fact that the free fin rays are used to locate food, the fin rays possess no taste buds (or olfactory receptors), and are innervated only by spinal nerves (Morrill, 1895). Whitear (1971) confirms that no specialized chemosensory end organs occur on the fin rays although numerous isolated chemosensory cells lie in the skin. The fin ray nerves emerge from the fused dorsal root ganglion of the second and third spinal roots (Herrick, 1907; also see Fig. 2). The nerves innervating the pectoral fin proper also emerge from this same ganglionic mass.

At the level of entrance of these nerve roots into the spinal cord, three major paired accessory spinal lobes occur on the dorsal aspect of the spinal cord. Herrick (1907) describes these accessory lobes as enlargements of the dorsal horn of the spinal cord. In addition, smaller swellings occur farther rostral in the spinal cord. Because the numbering system used by Herrick does not correspond to the patterns of lobulation observed in the live specimens (to which Herrick did not have access),

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Abbreviations: HRP, horseradish perioxidase; HY, Hanker-Yates peroxidase method; TMB, tetramethyl benzidine.

FIGURE 1. Photograph of a sea robin showing the free fin rays and pectoral fin. D, dorsal fin ray; M, middle fin ray; PF, pectoral fin; V, ventral fin ray. Approximately ⅔ life size.

the lobes have been renumbered in this work, according to the scheme indicated in Figure 2. The major accessory lobes are numbered 1–3, with number 1 being the most caudal. Accessory lobe 4 is a much smaller swelling located immediately rostral to the major accessory lobes. For reasons given elsewhere (T. Finger, in preparation) and below, the other swellings on the surface of the rostral spinal cord should not be considered homologous to the accessory lobes 1–4 described above and are not simple elaborations of the dorsal horn of the spinal cord.

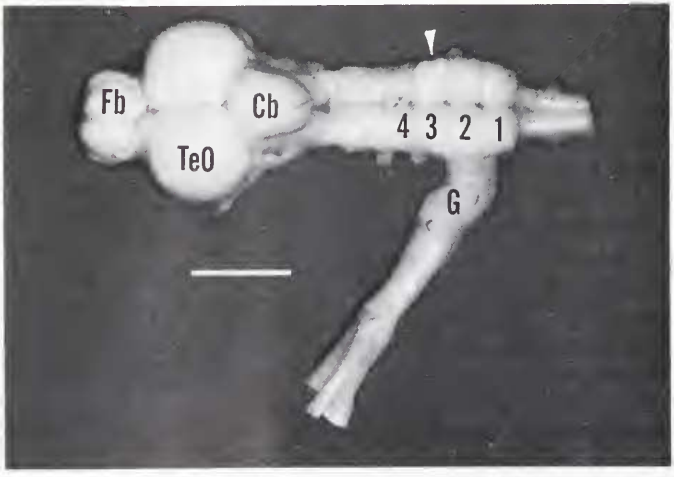

FIGURE 2. Photograph of the brain and spinal ganglion of a sea robin. White arrowhead indicates the sulcus of the accessory lobe wherein a blood vessel lies. 1, 2, 3, 4 indicate the accessory lobes of the spinal cord. Cb, cerebellum; Fb, forebrain; G, dorsal root ganglion for the fin ray nerves; TeO, optic tectum. Bar scale equals 5 mm.

The pathways and nuclei of the central nervous system involved in the spinal chemical sense have not been studied with modern anatomical techniques. This first in a series of papers on the common chemical sense in sea robins utilizes neuroanatomical tracing techniques to determine the representation of the pectoral fin and free fin rays in the rostral spinal cord. The pectoral fin nerves project to the minor accessory lobe (number 4) while the free fin rays project to the major accessory lobes (numbers 1–3).

Materials and Methods

Live sea robins (*Prionotus carolinus*) were obtained through the collection service at the Marine Biological Laboratory, Woods Hole, MA. The animals were fed pieces of squid and maintained in holding tanks supplied by running water.

Operations were carried out on fish which were anaesthetized with tricaine methane-sulfonate (MS-222). Initially, the animals were placed in seawater containing a 1:10,000 dilution of the drug. When opercular movements were barely perceptible or had ceased, the fish was transferred to an operation chamber which held the animal semirigidly by means of modelling clay blocks. The fish was covered with wet gauze and a recirculating pump supplied water to a tube inserted in the animal's mouth. The water in the operating chamber contained anaesthesia at a dilution of 1:20,000–1:40,000 depending on the anticipated duration of the procedure; higher concentrations were necessary for the longer operations. Following the surgery, the fish was placed in its home tank and revived by placing its mouth over the inlet tube for incoming seawater.

Horseradish peroxidase (HRP, Sigma Type VI) was used as an anterograde or transganglionic tracer. A 30–50% solution of HRP was prepared in a 1% solution of α-lysophosphatydal choline (lysolecithin). The tracer was applied in one of two fashions. For ganglionic injections, HRP paste was applied to the end of a size 00 insect pin which was then inserted repeatedly into the ganglionic mass (Finger, 1976). For applications to a peripheral nerve, the nerve was exposed and isolated on gelfoam. A small piece of gelfoam soaked in HRP was then placed on the cut end of the nerve. Fine forceps and an insect pin were used to divide the nerve into numerous fascicles which then were threaded through the HRP-gelfoam. In some cases, flattened sheets of styrofoam (from a hot-cup) were sandwiched around the nerve-gelfoam and the entire assembly glued (cyano-acrylate glue; histo-acryl or Crazy Glue®) back into place beneath the skin. The overlying skin was then sutured and glued to form a watertight covering.

A total of 12 fish was used in this study, but because some fish had two nerves labeled, one on each side, these animals represent 18 experiments. In four cases, the dorsal roots were labeled by intraganglionic injections of HRP. These cases provided the clearest labeling of the primary afferent terminals. The remaining 14 experiments entailed applications of HRP to the peripheral nerve as follows: ventral fin ray, three cases; middle fin ray, three cases; dorsal fin ray, four cases; superior pectoral fin nerve, two cases; inferior pectoral fin nerve, two cases.

Following survival times of 1–2 days for ganglionic injections (four cases) and 4–10 days for peripheral nerve injections (eight animals, 14 nerves), the fish were reanaesthetized and perfused through the conus arteriosus with 20 ml of saline followed by 50–100 ml of 4% glutaraldehyde in phosphate buffer (0.1 *M*, pH 7.2). The brain, rostral spinal cord, and, in some cases, spinal ganglia were removed from the animal, encased in gelatin (Finger, 1976) and fixed an additional 3–6 hours. The gelatin blocks were washed in phosphate buffer and stored overnight

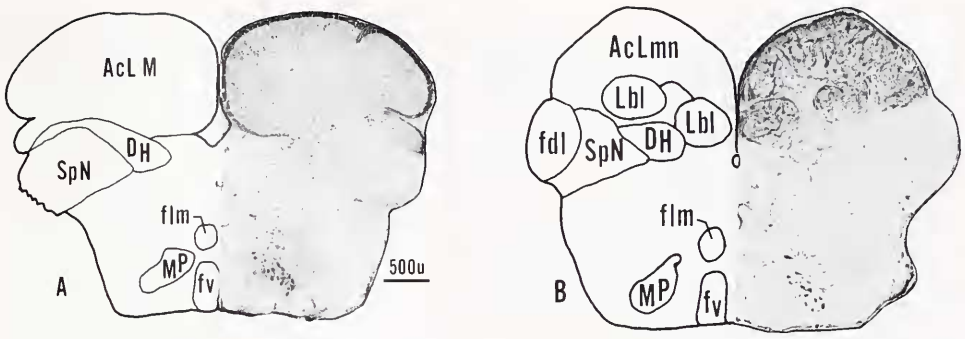

FIGURE 3. Photomicrographs and chartings of transverse sections through the accessory lobes. (A) Major accessory lobe. Note the prominent outer parvocellular layer surrounding the lobe. (B) Minor accessory lobe. The position of two lobules is indicated. AcLM, major accessory lobe; AcLmn, minor accesory lobe; DH, dorsal horn; Fdl, dorsolateral fasciculus; Flm, medial longitudinal fasciculus; fV, ventral fasciculus; Lbl, lobule; MP, segmental spinal motor neuron pool; SpN, spinal nerve root.

in buffer containing 10–20% sucrose. The tissue was sectioned at 35 μm in either the horizontal or transverse planes, on a freezing, sliding microtome. The sections were reacted for the presence of peroxidase by means of a modified Hanker-Yates (HY) method (Bell *et al.*, 1981) or by the tetramethylbenzidine (TMB) method of Mesulam (1978). In many cases alternate sections were reacted using the two different methods. The reaction product from the TMB method was visualized more easily, by darkfield microscopy, and the TMB reaction was slightly more sensitive. However, the HY method produced a less granular reaction product which better revealed fine structural details of the labeled fibers and cells.

RESULTS

Pattern of peripheral innervation. The three free fin rays are each innervated by a unique branch arising from the fused ganglion of the second and third spinal roots (Morrill, 1895; Herrick, 1907). The fin ray nerves form separate fascicles within 1 cm of the ganglion, somewhat dorsal to the pectoral fin proper.

Two other major nerves leave this same ganglionic mass to innervate the pectoral fin proper. A large nerve turns rostrally from the ganglion and enters the pectoral fin from its superior, or anterodorsal, aspect. This nerve is termed the superior pectoral fin nerve. The smaller nerve innervating the pectoral fin, the inferior pectoral fin nerve, travels with the fin ray nerves caudal to the pectoral fin but turns rostrally to innervate the inferior face of the pectoral fin. The fin ray nerves continue ventrally to reach the free fin rays. Immediately before entering the fin ray, a given fin ray nerve splits into two branches, one branch innervating the surface rostral to the cartilagenous core of the fin ray, and one branch innervating caudal to the cartilagenous core. No attempts were made in the present study to trace separate connections of the anterior and posterior branches of each fin ray nerve.

Structure of the accessory lobes. The major spinal accessory lobes (numbers 1–3) each are divided in half along their rostrocaudal axis by an indentation along the dorsal surface. This superficial groove often embraces a blood vessel. No similar division of the minor accessory lobe (lobe 4) occurs although blood vessels do run across the surface of this structure as well.

All of the accessory lobes contain an outer layer (approx. 40 μm thick) of small neurons and an inner zone of neurons mixed with neurophil (Fig. 3A). Golgi prep-

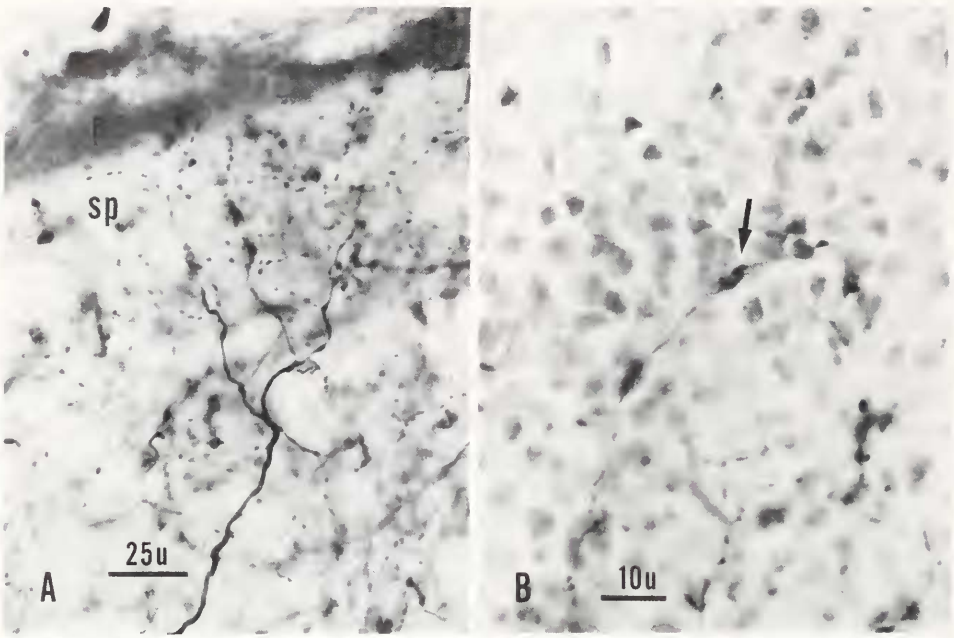

FIGURE 4. Photomicrographs of terminals and preterminal arborizations in the spinal accessory lobes following intraganglionic injection of HRP. Hanker-Yates reaction. (A) Numerous branches and *en passant* swellings occur in the subparvocellular layer (sp). p, parvocellular layer. (B) An apparent terminal among the small neurons of the parvocellular layer.

arations reveal that most dendrites of neurons in the accessory lobes are oriented radially. The detailed structure of the lobes will be discussed in a later paper (T. Finger, in preparation). The minor accessory lobe (lobe 4) comprises a number of lobules, each of which is surrounded by a parvocellular layer which extends inward from the surface of the lobe (Fig. 3B).

Primary afferent fibers. The morphology of the primary afferent fibers is revealed clearly by ganglionic injections of peroxidase. The nerve roots enter the spinal cord from its lateral aspect; in the case of lobe 4, the root has a slight rostral inclination as it penetrates the cord. A few root fibers terminate in the dorsal horn beneath the accessory lobes. The vast majority of the primary afferent fibers enter the accessory lobes from below and turn radially outward to terminate throughout the substance of the lobe. A given primary afferent fiber may branch repeatedly in its course outward through the lobe. Numerous terminal swellings occur throughout the lobe, however a heavier band of terminal arborization appears in the outer 50 μm of the neuropil of the lobe, *i.e.* immediately subjacent to the superficial parvocellular layer (Fig. 4A). A few terminal swellings are scattered amongst the somata in the parvocellular layer (Fig. 4B), but the bulk of the terminals and *en passant* swellings occur in the subjacent neuropil. This pattern of termination occurs in all the accessory lobes, minor as well as major.

Somatotopic organization. The central area of termination of each fin or fin ray nerve was determined by relying on transganglionic transport of HRP. This relatively fine, light labeling was revealed best by the TMB technique and darkfield microscopy (Fig. 5A) although the reaction product was clearly visible following reaction with the modified HY substrate.

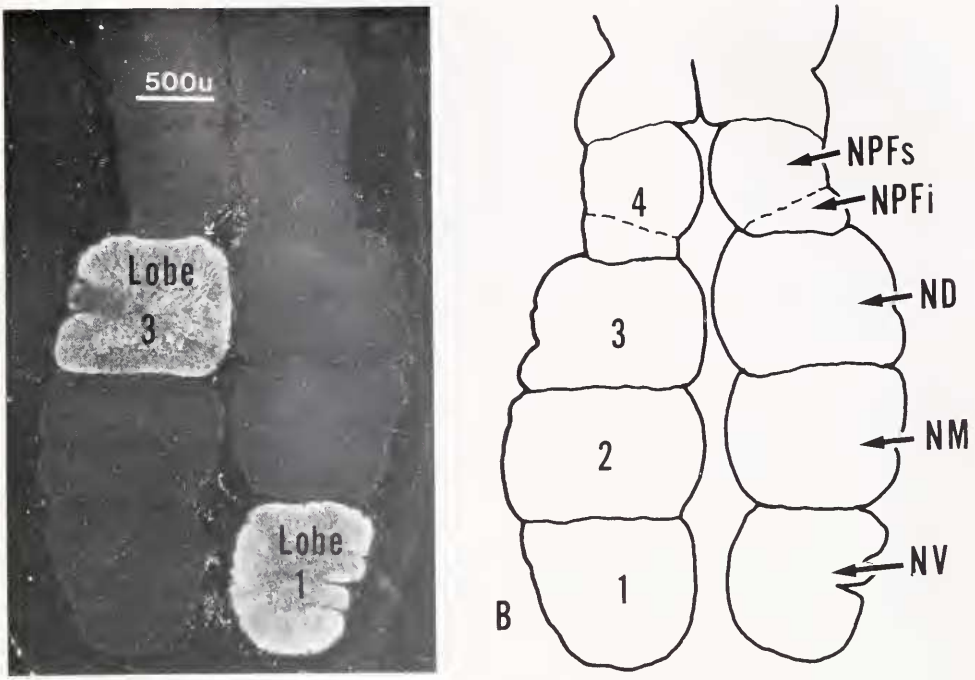

FIGURE 5. Horizontal section through the rostral spinal cord of a sea robin, anterior upward. (A) Darkfield photomicrograph from a case in which the dorsal fin ray nerve had been injected on the left side and the ventral fin ray nerve had been injected on the right side. Transganglionic transport of the peroxidase tracer shows in the photograph as a bright area (lobe 3 on the left and lobe 1 on the right). In these cases, no label appears in lobe 2 or the minor accessory lobe, above lobe 3. (B) Semischematic drawing of Fig. 5A showing the somatotopic representation of the various nerves in the rostral spinal cord. 1–4, accessory lobes; ND, dorsal fin ray nerve; NM, middle fin ray nerve; NPFi, inferior pectoral fin nerve; NPFs, superior pectoral fin nerve; NV, ventral fin ray nerve. Same scale as Fig. 5A.

Application of HRP to the nerve innervating the ventral fin ray resulted in labeling of terminals in lobe 1, the middle fin ray in lobe 2, and the dorsal fin ray in lobe 3 (Fig. 5). There was virtually no overlap between nerves in their projection onto the accessory lobes. The pectoral fin nerves terminate in the minor accessory lobe (lobe 4). The terminals from the inferior nerve occupy the caudolateral one-quarter of the lobe with the superior nerve terminals filling the remaining three-quarters of the structure (Fig. 5B). In summary, the central representation of the fin rays and pectoral fin is somatotopically organized. The ventral fin ray, farthest from the fin, is represented most posteriorly and the fin itself most anteriorly.

No primary afferent fibers ascend in the cord to reach the level of the caudal medulla. Therefore there does not appear to be a system in this species homologous to the dorsal columns found in amniote vertebrates.

DISCUSSION

Sea robins use their free fin rays to explore their surroundings. That the fin rays are chemoreceptive has been demonstrated both by behavioral and electrophysiological means (Scarrer *et al.*, 1947; Bardach and Case, 1965). Since the fin rays lack taste buds and are innervated only by spinal nerves, this chemosensitivity has been attributed to the common chemical sense (Parker, 1922). Therefore at

least some, if not most, of the fibers in the fin ray nerves mediate the common chemical sensibility. Compared to other spinal nerves, the pectoral fin and fin ray nerves are unique in terminating in the accessory lobes. Accordingly, the accessory lobes probably are involved in processing input from the common chemical sense.

The pectoral fin and fin rays are represented in a somatotopic order in the spinal cord. Somatotopy in a chemosensory system is not unique to this modality; a gustatory somatotopy has been described for catfish at the level of both the primary (Finger, 1976) and secondary (Finger, 1978) sensory nuclei.

One surprising result in this study is the order of the somatotopic map in the spinal cord. The ventral fin ray, which is also the most anterior part of the fin, is represented in the most posterior accessory lobe. The pectoral fin, which lies posterior (and dorsal) to the fin rays is represented farther anteriorly in the cord. If the pattern of innervation reflects the dermatome of origin in the embryo, then this implies that the anteroventral part of the fin, including the fin rays, arises posterior to the rest of the fin. If so, this further implies that the fin has rotated during embryogenesis such that the posterior edge of the fin in the embryo moves ventrally and rostrally during development so as to lie anterior and ventral to the rest of the fin in the adult.

The description of the accessory lobes given in this study is not identical to the descriptions offered by Morrill (1895), Ussow (cited in Morrill, 1895), or Herrick (1907). These authors describe six accessory lobes; the present report describes four. This difference is attributable to the more detailed study of intrinsic morphology and connections given in the present report. Four morphologically similar accessory lobes are described herein and they correspond to the accessory lobes 2–6 of Herrick (1907) which are identical to the five caudal lobes (unnamed) illustrated by Morrill (1895). Both of these authors divide accessory lobe 2 of this study into two parts on the basis of the sulcus in which the lobar blood vessel lies (see above). However, upon careful examination (see Fig. 2), all the major accessory lobes are marked by a similar sulcus. Since the portions of each lobe rostral and caudal to this sulcus are indistinguishable both in terms of morphology and connections, there is no apparent reason to separate these two halves of the same structure. In addition to the doubling of this central major accessory lobe (lobe 2), both Herrick and Morrill describe another accessory lobe lying rostral to the minor accessory lobe (lobe 4 of this study). This more rostral structure receives input predominantly from descending primary afferent fibers of the trigeminal nerve (T. Finger, unpub. obs.). As such, this structure is similar to the medial funicular nucleus described by Herrick (1906) and Finger (1976). Furthermore the morphology of this medial funicular nucleus in sea robins (Herrick's accessory lobe 1) is quite different from that of the accessory lobes proper. The medial funicular nucleus lacks the external cell layer which is characteristic of the accessory lobes. Accordingly, the present study does not include the medial funicular nucleus among the spinal accessory lobes.

The minor accessory lobe (lobe 4) receives input from the nerves innervating the pectoral fin which itself is supported by numerous fin rays. One possible explanation for the lobules in the minor accessory lobe is that each fin ray of the pectoral fin is represented in a single lobule. This conjecture needs to be tested by either finer anatomical or electrophysiological experiments. Furthermore, since the morphology of the minor accessory lobe is similar to the major lobes, the pectoral fin itself may be capable of chemoreception albeit with less sensitivity or discriminability than the free fin rays. Whitear (1971) reports that isolated chemosensory

cells are scattered throughout the epidermis of many, if not all, teleosts. Accordingly, the fin ray chemosense may represent a specialization of a spinal chemosense present in many vertebrates (Parker, 1922).

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